

THE ROLE OF ALCOHOL COMPOUNDS IN *CANDIDA ALBICANS* AND *CANDIDA DUBLINIENSIS* MORPHOGENESIS AND BIOFILM DEVELOPMENT

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Abstract

Over the last years, fungal diseases have become an increasing problem due to the use of broad-spectrum prophylaxes, chemotherapy and transplantation. Moreover, immunocompromised population (e.g., acquired immunodeficiency syndrome patients) is more likely than healthy one to develop fungal infections such as candidiasis. This caused a change in the spectrum of life-threatening pathogens, with *Candida* species such as *Candida dubliniensis* becoming increasingly important in medical microbiology. A recognized virulence trait of *Candida albicans*, shared by *Candida dubliniensis* is dimorphism, i.e., the ability to change between a filamentous and a yeast form. Insights into *Candida dubliniensis* virulence were recently stressed in a report using an animal model and tissue culture system, where filamentation ability and virulence were connected. Even though the very close phylogenetic relationship between both *Candida* species, *Candida dubliniensis* pathogenicity mechanisms are less well known.

The ability of microorganisms to sense and respond to extracellular factors has been investigated in prokaryotes. In fact, cell-cell signalling molecules were identified and shown to modulate biological functions such as virulence factors expression, biofilm formation or cell density. This has been an increasing area of research interest in eukaryotes, mainly concerning *Candida albicans* morphological regulation. Indeed, E,E-farnesol and tyrosol were identified as cell-cell signalling molecules in *Candida albicans* and have been demonstrated to regulate morphogenesis. In light of these findings, some authors suggested that discrepancies in the induction and kinetics of filamentation in *Candida albicans* and *Candida dubliniensis* should be due to different signals and pathways, even though to date these have not been recognized.

Aiming to contribute to a better understanding of *Candida albicans* and *Candida dubliniensis* pathogenicity, the main objectives of this study were to gain insights into morphogenesis control and biofilm development throughout chemical signals in these *Candida* species. In the last years several authors highlighted 3-methyl-1-butanol, 2-phenylethanol, 1-dodecanol and nerolidol as potential morphogenetic regulators to be evaluated.

Candida albicans CECT 1472 (Colección Española de Cultivos Tipo, Spain) and *Candida dubliniensis* CBS 7987 (Centraalbureau voor Schimmelcultures, Germany) standardized cells suspensions 1×10^6 yeast cells/ ml were prepared in RPMI 1640 medium. On one hand, the effect of specific alcohols in planktonic grown cells was assayed by adding such compounds to the culture medium at the

final concentrations: 23 mM and 46 μ M for 3-methyl-1-butanol, 500 μ M and 5 μ M, for 2-phenylethanol, 2 μ M and 2 nM for 1-dodecanol and 150 μ M, 1.5 μ M and 1.5 nM for nerolidol. Yeast cells were grown at 37 ° C on a mechanical shaker at 130 rpm for 12 h. After that, *Candida* cells morphology was assessed by light microscopy analysis and the percentage of filamentation calculated. *Candida* viability was assessed by spectrophotometric determinations. On the other hand, for biofilm experiments, *Candida albicans* and *Candida dubliniensis* biofilms were developed on the surface of microtiter plates and allowed to adhere for three hours at 37 °C, 130 rpm. To determine the effect of the specific alcohols on *Candida* biofilms formation and development, they were added at the beginning of the adhesion process or afterwards, respectively. The same concentrations of alcohols were used in planktonic and biofilm assays. Biofilms morphology was characterized by scanning electron microscopy. Quantitative measurements of biofilm formation were performed colorimetrically by a semiquantitative 2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide reduction assay.

Results obtained show that 3-methyl-1-butanol, 2-phenylethanol, 1-dodecanol and nerolidol regulate *Candida albicans* and *Candida dubliniensis* morphogenesis, repressing filamentation in a dose dependent fashion (Table 1).

TABLE 1. . Effect of alcohols on the morphology of planktonic grown *Candida albicans* CECT 1472 and *Candida dubliniensis* CBS 7987. Percentages of filamentation inhibition were calculated and expressed as mean \pm standard deviation.

Compound	Concentration	<i>Candida albicans</i> (% filamentation inhibition)	<i>Candida dubliniensis</i>
3-methyl-1-butanol	23 mM	95.82 \pm 2.96	92.38 \pm 2.14
	46 μ M	68.41 \pm 12.30	70.70 \pm 15.61
2-phenylethanol	500 μ M	95.32 \pm 1.23	91.54 \pm 10.66
	5 μ M	71.43 \pm 15.75	72.95 \pm 16.98
1-dodecanol	2 μ M	69.85 \pm 16.82	74.52 \pm 9.63
	2 nM	57.76 \pm 14.35	54.14 \pm 12.09
nerolidol	1.5 μ M	64.79 \pm 4.68	64.59 \pm 7.81
	1.5 nM	51.09 \pm 9.31	68.31 \pm 5.02

These compounds were noted to be active even at low concentrations (micromolar for 3-methyl-1-butanol and 2-phenylethanol and nanomolar 1-dodecanol and nerolidol). Moreover, it was observed that similar percentages of inhibition were obtained for both *Candida species*, suggesting that *Candida albicans* and *Candida dubliniensis* may share mechanisms of signalling and response to these alcohols. The significance of these compounds in biofilm formation and development will be discussed.

These studies will contribute to a better understanding of the morphogenesis and biofilm regulation networks in *Candida albicans* and *Candida dubliniensis* throughout specific alcohols signalling.